Trapping Alfalfa Looper Moths (Lepidoptera: Noctuidae) with Single and Double Component Floral Chemical Lures

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Environ. Entomol. 30(4): 667-672 (2001)

ABSTRACT Both sexes of the alfalfa looper moth, *Autographa californica* (Speyer), were captured in traps baited with chemicals and combinations of chemicals that are odorants from "moth-visited" flowers. When presented alone, phenylacetaldehyde was strongly attractive and benzyl acetate was more weakly attractive to alfalfa looper moths. Few alfalfa looper moths were captured in traps baited with *cis*-jasmone, linalool, phenethyl alcohol, benzyl acetate or benzyl alcohol. In a comparison of varied amounts of phenylacetaldehyde, accomplished by varying the diameter of the hole in the lid of vial dispensers, greatest captures of alfalfa looper moths occurred with the largest hole size tested, 6.3 mm in diameter. Catches of alfalfa looper moths were enhanced when phenylacetaldehyde was presented with *cis*-jasmone and when benzyl acetate was presented along with benzaldehyde, compared with these chemicals presented singly.

KEY WORDS alfalfa looper, Autographa californica, attractant, trap, phenylacetaldehyde

THE FLOWERS OF a number of plant species produce odorants that are strongly attractive to moths. For example, Cantelo and Jacobson (1979a) determined that phenylacetaldehyde emitted by the bladder flower, Araujia sericofera Brot., attracted numerous species of moths to that plant. Chemicals found in the odor of flowers of Abelia grandiflora (Andre) (Haynes et al. 1991) and from flowers of night blooming jessamine, Cestrum nocturnum L. (Heath et al. 1992a), are attractive to cabbage looper moths, Trichoplusia ni (Hübner). The pink bollworm, Pectinophora gossypiella (Saunders), is attracted to the odor of cotton flowers, Gossipium hirsutum L., possibly as a foodfinding behavior (Wiesenborn and Baker 1990). Corn earworm moths, Helicoverpa zea (Boddie), are attracted to the odors of flowers of Japanese honeysuckle, Lonicera japonica (Pair and Horvat 1997), and Gaura suffulta (Engelm.) (Beerwinkle et al. 1996), whereas the tobacco budworm moth, Heliothis virescens (F.) is attracted to volatile chemicals of cotton flowers (Tingle and Mitchell 1992).

Pestiferous members of the Noctuidae subfamily Plusiinae, often referred to as loopers, are noteworthy in commonly feeding at flowers (Eichlin and Cunningham 1978) and in their attraction to chemicals that have been isolated from flowers. *Trichoplusia ni* moths are often seen at flowers (Grant 1970, Heath et al. 1992b) and this species is attracted to phenylacetaldehyde, benzaldehyde, benzyl acetate, 2-phenylethanol, (phenethyl alcohol), and other com-

We report here results of experiments to determine the response of alfalfa looper moths to a series of volatile chemicals reported as odorants from flowers (bladder flower, night-blooming jessamine, Japanese honeysuckle, *Gaura* species, and *A. grandiflora*) that are thought to be attractive to moths, including Plusinae moths (Cantelo and Jacobson 1979a, Haynes et al. 1991, Heath et al. 1992a, Pair and Horvat 1997, Schlotzhauer et al. 1996). These floral chemicals were used to bait traps, and the presence of moths in traps was considered to be evidence of moth attraction to those chemicals. These results not only demonstrate alfalfa looper attraction to particular floral compounds, but provide a means of luring and trapping both sexes of this pest moth.

Materials and Methods

General. The Universal Moth Trap (Agrisense, Fresno, CA), here called the Unitrap, was used in all

pounds that are present in the volatiles of attractive flowers (Smith et al. 1943, Haynes et al. 1991, Heath et al. 1992a, Lopez et al. 2000). Smith et al. (1943) reported the trapping of other species of Plusiinae with these same chemicals; the soybean looper *Pseudoplusia includens* (Walker), clover looper *Rachiplusia ou* (Guénee), *Autographa oxygramma* (Geyer), *Argyrogramma verruca* (F.), *Argyrogramma basigera* (Walker), and *Autographa biloba* (Stephen). In the western United States, *Autographa californica* (Speyer) is an abundant and polyphagous pest of numerous crops (Eichlin and Cunningham 1978). We hypothesized that this moth, like *T. ni* and other Plusiinae, is attracted to flowers by olfactory orientation to particular floral odorants.

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experiments. A 6-cm² piece of Vaportape (Hercon, Emigsville, PA) was placed in the bottom of each trap to kill captured insects. Vaportape was replaced every 2 wk. Polypropylene vials (8 ml) (Nalgene 2006–9025, Fisher, Pittsburg, PA) were used as attractant dispensers for all tests. Two cotton balls were placed in the bottom of each vial and each vial was then loaded with 5 ml of a test chemical. A 3.0-mm hole was drilled through each vial lid to permit the release of the test chemical from the vials (unless otherwise indicated). Vials were suspended upright within the bucket of the Unitrap by a wire. Traps were hung at a height of ≈ 0.7 m from either fence wire or were suspended from stakes placed in the ground along the periphery of commercial fields of alfalfa (Medicago sativa) and corn (Zea mays). Trapping sites were near Prosser in Benton County, 20 km east of Moxee in Yakima County, and near Mattawa in Grant County, all in the state of Washington. Insects captured in traps were placed in labeled plastic bags for transport to the laboratory where moths were identified and sorted by sex.

Three different experiments were conducted to assess alfalfa looper moth response to individual floral odorants, to assess the relationship between the amounts of evaporated phenylacetaldehyde and moth captures, and then to assess moth responses to 2-component combinations of the same floral odorants. Each experiment was conducted for 2 wk. Traps were checked and cleaned and their position was randomized twice per week, providing 20 replicates per treatment for each of the field tests. For all three experiments, numbers of males and females captured were similar among attractive treatments and numbers for each sex were combined for statistical analyses of trap catch data.

Single Floral Odorants. Seven chemicals were tested in comparison to each other and to an unbaited trap. The chemicals tested were phenylacetaldehyde, phenethyl alcohol, benzyl acetate, benzyl alcohol, benzaldehyde, and cis-jasmone, purchased from Aldrich Chemicals (Milwaukee, WI) and a racemic mixture of linalool purchased from Fluka (Milwaukee, WI). A randomized complete block design was used, with each chemical and an unbaited trap included within each block. Five such blocks were set up along north-south running fences adjacent to fields of alfalfa. Traps were hung on the fence and were separated by 10 m. This experiment was conducted at the Prosser and Moxee site for 2 wk in late May and early June 2000 and again at the Mattawa site for 2 wk in late June and early July of 2000.

Data from all treatments were analyzed by an analysis of variance (ANOVA) and means were compared using Tukey's test to determine differences among chemical treatments.

Phenylacetaldehyde Dose–Response. Six release rates of phenylacetaldehyde were compared for their attractiveness to alfalfa looper moths, with the release rates of phenylacetaldehyde from vials varied by altering the diameter of the hole drilled into the vial lid. Polypropylene vials were loaded with 5 ml of phenylacetaldehyde and holes of 0.00, 0.5, 1.0, 1.5, 3.0, and 6.3

mm in diameter were made in the vial lids. A randomized complete block design was used in this experiment also, with five blocks. Traps were placed along a north-south running fence line adjacent to alfalfa fields, at the Moxee site, with traps 10 m between traps.

Trap catch data were subjected to a regression analysis to determine if there was a significant relationship between vial hole diameter and numbers of alfalfa looper moths captured.

Two Component Blends. In a series of five tests, all 2-component combinations of the seven chemicals (phenylacetaldehyde, phenethyl alcohol, linalool, benzyl acetate, benzyl alcohol, benzaldehyde, and *cis*-jasmone) were assessed in comparison to phenylacetaldehyde alone (as a positive control), to one of the two chemicals alone, and to an unbaited trap (negative control). All chemicals were dispensed from 15-ml polypropylene vials with 10 ml of a chemical placed in each vial and each vial having a 3.0-mm-diameter hole in the lid. For 2-chemical combinations, two vials then were used to bait the trap; each baited with one chemical of the combination. All five tests in this experiment were conducted along weedy field borders, in the vicinity of alfalfa fields near Mattawa, WA

In the first 2-component test there were eight treatments; an unbaited trap, a trap baited with phenylacetaldehyde alone, and traps baited with phenylacetaldehyde together with either phenethyl alcohol, linalool, benzyl acetate, benzyl alcohol, benzaldehyde, or *cis*-jasmone. In the second 2-component test there were eight treatments; an unbaited trap, a trap baited with phenylacetaldehyde alone, a trap baited with *cis*-jasmone alone, and traps baited with *cis*-jasmone together either with phenethyl alcohol, linalool, benzyl acetate, benzyl alcohol, or benzaldehyde. In the third 2-component test there were seven treatments; an unbaited trap, a trap baited with phenylacetaldehyde, a trap baited with benzyl acetate alone, and traps baited with combinations of benzyl acetate and either, benzyl alcohol, benzaldehyde, linalool, or phenethyl alcohol. In the fourth 2-component test there were six treatments; an unbaited trap, a trap baited with phenylacetaldehyde, a trap baited with benzyl alcohol, and traps baited with the combinations of benzyl alcohol with either benzaldehyde, linalool, or phenethyl alcohol. In the fifth 2-component test there were eight treatments; an unbaited trap, a trap baited with phenylacetaldehyde, a trap baited with benzaldehyde, a trap baited with linalool, a trap with phenethyl alcohol, a trap baited with phenethyl alcohol and linalool, and traps baited with benzaldehyde and either linalool or phenethyl alcohol.

Measurements were made of release rates of phenylacetaldehyde from polypropylene vials, using a volatile collection system consisting of a compressed nitrogen source, charcoal filter, flowmeter, aeration chamber, and trap. Four 8-ml vials with 3-mm holes in the lids and four 3-ml vials with 6.3-mm holes in lids were loaded with 5 ml phenylacetaldehyde on cotton balls. Two days after vials were loaded, volatile collections were made. Individual vials were placed in a 500-ml glass aeration chamber through which metered

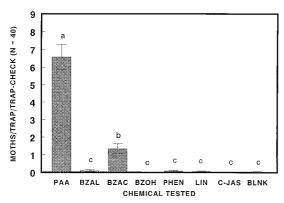


Fig. 1. Mean \pm SE numbers of alfalfa looper moths captured in traps baited with seven different floral odorants and in unbaited traps (BLNK). Chemicals tested were phenylacetaldehyde (PAA), benzaldehyde (BZAL), benzyl acetate (BZAC), benzyl alcohol (BZOH), phenethyl alcohol (PHEN), linalool (LIN), and cis-jasmone (C-JAS). Bars with the same letter are not significantly different by Tukey's test, at P < 0.05.

and charcoal-purified $\rm N_2$ was passed at 500 ml/min. After the volatile collection system was purged with $\rm N_2$ for 30 min, $\rm N_2$ was passed through the aeration chamber containing the phenylacetaldehyde vial and through a trap containing 30 mg of SuperQ sorbent. SuperQ traps were washed with 3 aliquots of 300 ml methylene chloride, which was then analyzed using a Hewlett–Packard (Palo Alto, CA) 5890 gas chromatograph with a 60 m J & W DB-1 capillary column (J & W Scientific, Folsum, CA). Quantification of phenylacetaldehyde in extracts of SuperQ traps was made by comparison to phenylacetaldehyde standards.

Within each of the five 2-component tests, trap catch data were subjected to an ANOVA and differences among means were determined by Tukey's test.

Voucher specimens of *A. californica* are deposited in the M. T. James Entomological Collection in the Department of Entomology, Washington State University, Pullman, WA.

Results

Single Floral Odorants. Significantly higher numbers of alfalfa looper moths were captured in traps baited with phenylacetaldehyde and in traps baited with benzyl acetate compared with unbaited traps (Fig. 1). A few alfalfa looper moths were captured in traps baited with other chemicals, but these numbers were not significantly higher than numbers of moths in unbaited traps. Numbers of alfalfa looper moths captured in traps baited with phenylacetaldehyde were significantly greater than the numbers captured in traps baited with benzyl acetate. A total of 318 alfalfa looper moths was trapped in this experiment (150 females and 168 males).

Phenylacetaldehyde Dose–Response. Alfalfa looper moths were captured in traps for all treatment categories, but captures increased with increases in vial

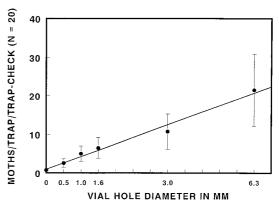


Fig. 2. Mean \pm SE numbers of alfalfa looper moths captured in traps baited with phenylacetaldehyde dispensed from polypropylene vials with holes of different diameters for varying of phenylacetaldehyde release.

hole diameter, indicating increased attractiveness with increased phenylacetal dehyde release rate (Fig. 2). There was a significant regression of moth captures with vial hole diameter $(r^2=0.93,\,\mathrm{df}=5,\,P<10^{-4},\,\mathrm{Y}=1.16+3.16\mathrm{X},\,\mathrm{where}\,\,\mathrm{Y}$ is the number of moths captured and X is vial hole diameter in mm). Greatest numbers of alfalfa looper moths were captured in traps baited with vials of phenylacetal dehyde possessing a 6.3-mm-diameter hole in the lid, the largest hole size tested. A total of 536 alfalfa looper moths was captured in this experiment (247 females and 289 males).

Two-Component Blends. In the first 2-component test (Fig. 3A) the numbers of alfalfa looper moths captured in traps baited with the combination of phenylacetaldehyde plus *cis*-jasmone were significantly greater than the numbers captured in traps baited with phenylacetaldehyde alone. No other combinations of chemicals resulted in catches of moths significantly greater than in traps baited with phenylacetaldehyde alone. All chemical treatments in this test resulted in significantly higher numbers of moths captured, compared with unbaited traps. A total of 354 alfalfa looper moths was captured in this test (110 females and 244 males).

In the second 2-component test (Fig. 3B), significantly higher numbers of alfalfa looper moths were captured in traps baited with *cis*-jasmone plus benzyl acetate and in traps baited with phenylacetaldehyde alone, compared with unbaited traps. Numbers of moths trapped with phenylacetaldehyde were significantly greater than with the combination of *cis*-jasmone and benzyl acetate. There was no indication of any synergy or enhancement of moth attraction with *cis*-jasmone released along with other chemicals in this test. A total of 170 alfalfa looper moths was captured in this test (78 females and 92 males).

In the third 2-component test (Fig. 3C), significantly higher numbers of alfalfa looper moths were captured in traps baited with all chemical treatments, compared with unbaited traps. The combination of benzyl acetate and benzaldehyde resulted in in-

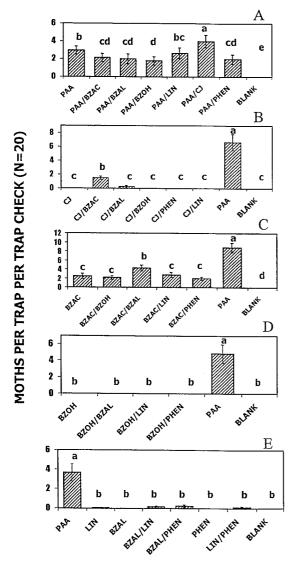


Fig. 3. Mean \pm SE numbers of alfalfa looper moths captured in traps baited with double component floral lures, compared with unbaited traps, traps baited with phenylacetaldehyde, and traps baited with one of the blend components. Chemicals tested were phenylacetaldehyde (PAA), benzaldehyde (BZAL), benzyl acetate (BZAC), benzyl alcohol (BZOH), phenethyl alcohol (PHEN), linalool (LIN), and *cis*-jasmone (CJ). Within each of the five tests, bars with the same letter are not significantly different by Tukey's test at P < 0.05.

creased trap catches compared with benzyl acetate alone. All 2-component combinations in this test however were inferior to phenylacetaldehyde alone. A total of 446 alfalfa looper moths was captured in this test (239 females and 207 males).

In the fourth 2-component test (Fig. 3D), moths were captured only in traps baited with phenylacetaldehyde. Alfalfa looper moths were not captured in traps baited with benzyl alcohol, or in traps baited

with benzyl alcohol in combination with other chemicals. A total of 97 alfalfa looper moths was captured in this test (40 females and 57 males).

In the fifth and last 2-component test (Fig. 3E), greatest captures of moths were in traps baited with phenylacetaldehyde alone. Numbers of alfalfa looper moths in traps baited with all other chemicals and chemical combinations were not significantly higher than in unbaited traps. A total of 44 alfalfa looper moths was captured in this experiment (6 females and 38 males).

Volatile collections made on 8-ml vials loaded with phenylacetal dehyde yielded 43.4 ± 2.0 and 97.2 ± 4.2 micrograms phenylacetal dehyde per hour for vials with 3- and 6.3-mm-diameter holes, respectively.

Discussion

Of the seven chemicals evaluated, phenylacetaldehyde and benzyl acetate were attractive to the alfalfa looper moth when presented singly. This attractiveness was indicated by significantly higher numbers of moths captured in traps baited with dispensers releasing these compounds, compared with unbaited traps. Alfalfa looper moths were not captured in significant numbers in traps baited with *cis*-jasmone, benzaldehyde, benzyl alcohol, linalool, or phenethyl alcohol.

Phenylacetaldehyde is attractive to other moths, particularly species of Plusiinae. Smith et al. (1943) trapped large numbers of the plusiine noctuids T. ni, P. includens, A. oxygramma, A. verruca, A. basigera, and A. biloba in Louisiana with phenylacetaldehyde. Creighton et al. (1973) trapped T. ni, P. includens, T. oxygramma, A. verruca, and A. basigera with phenylacetaldehyde in South Carolina. Cantelo and Jacobson (1979b) baited traps in Maryland with phenylacetaldehyde and captured numbers of several economically important moth pests, including P. includens. Trichoplusia ni females were attracted to this chemical also in flight tunnel assays (Havnes et al. 1991, Landolt et al. 1991, Heath et al. 1992). It is not surprising then that phenylacetaldehyde is attractive to A. californica moths.

Release rates of phenylacetaldehyde from the vials with 3-mm holes used in most of these experiments and from vials with the largest hole size tested (6.3 mm) were large (43 and 97 μ g phenylacetaldehyde per hour, respectively) in comparison to measurements made of phenylacetaldehyde released from flowers. Haynes et al. (1991) found 4-5 μ g phenylacetaldehyde emitted per day by A. grandiflora flowers and Heath et al. (1992) reported 52.8 \pm 4.5 ng phenylacetaldehyde per hour from flowers of C. nocturnum. However, shrubs of either of these species may possess numerous flowers emitting phenylacetaldehyde and other odorants. These measurements provide a basis for comparison with other methods of formulating and dispensing floral attractants.

Benzyl acetate is also reported to be attractive to several other moths. This compound was found in the volatiles of flowers of *C. nocturnum* and was shown to be attractive to *T. ni* moths in a flight tunnel (Heath

et al. 1992a). Smith et al. (1943) captured large numbers of *Autographa* (sensu latu) moths (=several Plusiinae genera and species) in traps baited with benzyl acetate.

The other chemicals that we tested are also reported to be part of the odors of flowers visited by moths. Benzaldehyde, benzyl alcohol, phenethyl alcohol, and *cis*-jasmone are odorants of flowers known to be attractive to other Plusiinae moths (Haynes et al. 1991, Heath et al. 1992a, Pair and Horvat 1997). Linalool is a plant chemical (Masada 1976) and flower odorant (Pair and Horvat 1997) as well as the main component of the male pheromone of *T. ni* (Heath et al. 1992b). There are no prior reports of alfalfa looper moth responses to any of these chemicals or other floral odorants.

The combination of phenylacetaldehyde and cisjasmone resulted in greater captures of alfalfa looper moths. Because cis-jasmone alone is not attractive, this suggests that the chemical has some enhancement effect on moth responses to phenylacetaldehyde. The possibility that these two chemicals are enhancing or synergistic in attracting alfalfa looper moths calls for additional study. Pair and Horvat (1997) found the T. ni moth attraction to the combination of phenylacetaldehyde with *cis*-jasmone is greater than to either chemical alone. Numbers of alfalfa looper moths in traps baited with the combination of benzyl acetate and benzaldehyde were greater than in traps baited with either chemical alone. There were no other 2-component combinations providing enhanced captures of moths. Overall, the strongest chemical attractant tested was phenylacetaldehyde. In the single chemical test, phenylacetaldehyde was the best attractant for the alfalfa looper and in four of the five 2-component tests, there were no chemicals or chemical blends tested that were comparable to phenylacetaldehyde as a lure.

The general lack of response of alfalfa looper moths to linalool, phenethyl alcohol, benzyl alcohol, and cis-jasmone, either presented singly or in blends, does not entirely exclude these chemicals as feeding attractants. They may be co-attractive when presented with other untested chemicals or in more complex blends, or when released in different amounts. Linalool was tested as a racemic mixture, with both enantiomers present. Possibly one or the other enantiomer is active when presented in a purer form, such as occurs when d-linalool is presented with p- and mcresol as the male pheromone of the cabbage looper (Heath et al. 1992b).

As with other moths, both sexes of the alfalfa looper are attracted to these floral chemicals. The overall sex ratio, for all experiments combined, was 45.5% females to 54.5% males. The sex ratio of cabbage looper moths captured by Creighton et al. (1973) in traps baited with phenylacetaldehyde was 55.6% females to 44.4% males. Pair and Horvat (1997) found that both sexes of several species of moths, including the cabbage looper and soybean looper, are attracted to several combination of chemicals isolated from Japanese honey-suckle floral odors. The response of females of pest

moth species to these odors suggests possibilities both for monitoring of female activities and for control of alfalfa loopers through mass trapping of adults or the use of a poison bait.

One concern with the use of chemical lures comprised of flower odorants is their attractiveness to nontarget insects. Insects of concern include bees, wasps, and butterflies. In these experiments, nontarget insects generally were not identified and quantified. However, it was obvious that large numbers of bees, including species of Bombus, sphecid wasps and coccinellid beetles were captured in these traps, potentially in response to chemicals tested. Meagher and Frank (1998) reported capturing numbers of a sphecid wasp, Larra bicolor F. in traps baited with phenylacetaldehyde or the combination of phenylacetaldehyde and sex pheromones of Spodoptera spp. in Florida. This wasp is an important predator introduced into Florida for biological control of pest mole crickets. Subsequently, Meagher and Mitchell (1999) reported the trapping of other Sphecoidea in traps baited with phenylacetaldehyde. Efforts to develop applications of these lures should take such problems into account. Perhaps chemical lures, trap designs, and trap placement can be designed to minimize impact on beneficial/desirable insects while still maintaining efficacy for pest insects.

Acknowledgments

Technical assistance was provided by J. Alfaro, L. Biddick, J. Brumley, P. Chapman, B. Cleveland, and J. Dedlow. We thank C. Martin (Del Monte, Inc.) and R. Martinez for use of their fields. We thank S. Halstead of EPA for her help in facilitating this project. Helpful comments to improve the manuscript were made by L. P. S. Kuenen, J. Hansen, K. F. Haynes, and E. R. Mitchell. This work was supported in part by the Columbia Basin Vegetable Processors Association, by USDA, NRI Research Career Enhancement Grant 80.1, by a Food Quality Protection Act Award from U.S. EPA, and by a sabbatical leave granted to H.C.R. from Oral Roberts University.

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Received for publication 22 November 2000; accepted 12 March 2001.